Amendments to the Claims

Pursuant to 37 C.F.R. §1.121 the following is a complete listing of the claims of the present application. This listing replaces all prior listings of claims.

Claims 1-56 [Canceled]

57. [currently amended] A method for sequencing one or more target nucleic acids present in one or more biological samples, said method comprising the steps of:

(a) deriving from one or more biological samples the one or more target nucleic acids;

(b) subjecting the one or more target nucleic acids obtained from step (a) to two or more separate base-specific, sequence-specific or site-specific complementary cleavage reactions, wherein each cleavage reaction generates a non-ordered set of fragments;

(c) analyzing the sets of non-ordered fragments obtained from step (b) by mass spectrometry; and,

(d) performing a systematic computational analysis on the mass spectra obtained from step (c) to analyze the sequence of said target nucleic acid;

wherein said complementary cleavage reactions ~~refer to~~ comprise target nucleic acid digestions characterized by varying specificity and/or to digestion of alternative forms of the target sequence.

58. [currently amended] The method according to claim 57 wherein the one or more biological samples are derived from an organism selected from the group consisting of eukaryotes, prokaryotes, and viruses.

59. [previously presented] The method according to claim 57 wherein the one or more target nucleic acids are selected from the group consisting of single stranded DNA, double stranded DNA, cDNA, single stranded RNA, double stranded RNA, DNA/RNA hybrid, and DNA/RNA mosaic nucleic acid.

60. [previously presented] The method according to claim 57 wherein one or more target nucleic acids are derived by one or more consecutive amplification procedures selected from the group consisting of in vivo cloning, polymerase chain reaction (PCR), reverse transcription followed by the polymerase chain reaction (RT-PCR), strand displacement amplification (SDA), and transcription based processes.

61. [currently amended] The method according to claim 60 wherein the one or more amplified target nucleic acids are transcripts generated from a single stranded or a double stranded target nucleic acid by a process comprising the steps of:

(a) ~~linking~~ operatively linking a transcription control sequences to the one or more target nucleic acids; and

(b) transcribing one or both strands of the one or more target nucleic acid of step a) using one or more RNA polymerases that recognize the transcription control sequence on the one or more target nucleic acids.

62. [currently amended] The method according to claim 61 wherein said transcriptional control sequences ~~are~~ is operatively linked to one or more target nucleic acids by PCR amplification using a primers that incorporates the transcriptional control sequences as a 5'-extensions.

63. [currently amended] The method according to claim 61 wherein the transcription control sequence is selected from the group consisting of a eukaryotic transcription control sequences, a prokaryotic transcription control sequences, and a viral transcription control sequences.

64. [previously presented] The method according to claim 63 wherein the prokaryotic transcription control sequence is selected from the group consisting of T3, T7, and SP6 promoters.

65. [previously presented] The method according to claim 64 wherein the RNA polymerases which utilize the T3, T7, or SP6 promoters are either wild type or mutant

RNA polymerases, the mutant polymerases being capable of incorporating into the transcript non-canonical substrates with a 2'-deoxy, 2'-O-methyl, 2'-fluoro or 2'-amino substituent.

66. [currently amended] The method according to claim 65 wherein the mutant RNA polymerase is either T7 mutant polymerase or SP6 mutant polymerase.

67. [previously presented] The method according to claim 57 wherein the derived target nucleic acid incorporates one or more nucleosides that are modified on the base, the sugar, and/or the phosphate moiety, wherein the modifications alter the specificity of cleavage by the one or more cleavage reagents and/or the mass and/or the length of the cleavage products.

68. [currently amended] The method according to claim 67 wherein the modification is introduced through the enzymatic incorporation of a modified deoxynucleoside triphosphates, a modified ribonucleoside triphosphates, and/or a modified dideoxynucleoside triphosphates; or wherein the modification is introduced chemically, or wherein the modification is introduced through a combination of both methods.

69. [previously presented] The method according to claim 67 wherein the modification consists of a 2'-deoxy, 2'-O-methyl, 2'-fluoro or 2'-amino substituent on the nucleotide triphosphates.

70. [previously presented] The method according to claim 67 wherein the modification consists of phosphorothioate internucleoside linkages or phosphorothioate internucleoside linkages further reacted with an alkylating reagent.

71. [previously presented] The method according to claim 67 wherein the modification consists of a methyl group on C5 of the uridine-5'-monophosphate subunits.

72. [previously presented] The method according to claim 67 wherein the modification consists of nucleotides that incorporate alternative isotopes.

73. [previously presented] The method according to claim 57 wherein the one or more target nucleic acids of step (a) are purified prior to cleavage.

74. [previously presented] The method according to claim 73 wherein said purification is achieved through immobilization or by chromatography.

75. [previously presented] The method according to claim 57 wherein the complementary cleavage reactions are selected from the group consisting of enzymatic cleavage, chemical cleavage, and physical cleavage.

76. [previously presented] The method according to claim 75 wherein the complementary cleavage reactions are characterized by a relaxed mono-nucleotide, mono-nucleotide, relaxed di-nucleotide, or di-nucleotide specificity.

77. [previously presented] The method according to claim 75 wherein the one or more target nucleic acids are subjected to chemical digestion reaction consisting of treatment with alkali or with reagents used in the Maxam & Gilbert sequencing method.

78. [previously presented] The method according to claim 75 wherein the one or more target nucleic acids are subjected to enzymatic cleavage reaction using one or more enzymes selected from the group consisting of endonucleases and exonucleases.

79. [previously presented] The method according to claim 78 wherein the one or more target nucleic acids are subjected to enzymatic cleavage reaction using one or more endonucleases, selected from the group consisting of restriction enzymes, RNA endonucleases, DNA endonucleases and non-specific phosphodiesterases.

80. [previously presented] The method according to claim 79 wherein the one or more endonucleases are one or more selective or non-selective RNA endonucleases, selected from the group consisting of the G-specific T1 ribonuclease, the A-specific U2 ribonuclease, the A/U specific phyM ribonuclease, the U/C specific ribonuclease A, the C-specific chicken liver ribonuclease (RNaseCL3) and cusativin, non-specific RNase-I, and pyrimidine-adenosine preferring RNases isolated from E. coli, Enterobacter sp., or Saccharomyces cerevisiae.

81. [previously presented] The method according to claim 57 wherein the one or more target nucleic acids are phosphorothioate-modified single stranded DNA or RNA, and wherein the cleavage reactions are performed with the nuclease P1.

82. [previously presented] The method according to claim 57 wherein the one or more target nucleic acids are mosaic RNA/DNA nucleic acids or modified mosaic RNA/DNA nucleic acids, prepared with mutant polymerases, and wherein the cleavage reagents are RNA endonucleases, DNA endonucleases or alkali.

83. [previously presented] The method according to claim 57 wherein the one or more target nucleic acids are transcripts, modified transcripts, mosaic RNA/DNA transcripts or modified mosaic RNA/DNA transcripts, prepared with wild type or mutant RNA polymerases, and wherein the cleavage reagents are one or more selective or non-selective RNA endonucleases or alkali.

84. [currently amended] The method according to claim 57 wherein the one or more target nucleic acids are mosaic RNA/DNA transcripts that incorporate either dCMP, dUMP or dTMP, prepared with mutant T7 or SP6 polymerase, and wherein the cleavage reagent is a pyrimidine-specific RNase.

85. [currently amended] The method according to claim 57 ~~wherein~~ further comprising ion-exchange chromatographic purification of the set of non-ordered fragments of step (b) ~~is additionally purified using an ion exchange beads~~.

86. [previously presented] The method according to claim 57 wherein the set of non-ordered fragments of step (b) is spotted onto a solid support.

87. [previously presented] The method according to claim 86 wherein said solid support is chosen from a group consisting of solid surfaces, plates and chips.

88. [previously presented] The method according to claim 57 wherein the mass spectrometric analysis of the nucleic acid fragments is performed using a mass spectrometric method selected from the group consisting of Matrix-Assisted Laser

Desorption/Ionization-Time-of-flight (MALDI-TOF), Electrospray-Ionization (ESI), and Fourier Transform-Ion Cyclotron Resonance (FT-ICR).

89. [currently amended] The method according to claim 57, wherein said method is used for re-sequencing of one or more target nucleic acids for which a reference nucleic acid sequence is known; said method comprising an additional step wherein the one or more mass spectra of the non-ordered fragments obtained in step c) are compared with the known or predicted mass spectra for a reference nucleic acid sequence, and deducing therefrom, by systematic computational analysis, all or part of the nucleotide sequence of the one or more target nucleic acids, and comparing the deduced nucleic acid sequence with the reference nucleic acid to determine whether the one or more target nucleic acids have the same sequence or a different sequence from the reference nucleic acid.

90. [previously presented] The method according to claim 89 wherein the nucleic acid sequence difference that is determined is a deletion, substitution, insertion or combinations thereof.

91. [previously presented] The method according to claim 90 wherein the nucleic acid sequence difference is a Single Nucleic Polymorphism (SNP).

92. [previously presented] The method according to claim 89 wherein said method identifies known as well as unknown nucleotide sequence variations of said one or more target nucleic acids present in said one or more biological samples.

93. [previously presented] The method according to claim 92 wherein determination of said known or unknown nucleotide sequence variations allows the identification of the various allelic sequences of a certain region/gene, the scoring of disease-associated mutations, the detection of somatic variations, or studies in the field of molecular evolution.

94. [previously presented] The method according to claim 57 wherein the spectra obtained for one or more target nucleic acids are compared with the mass spectra predicted for a plurality of reference nucleic acids thereby identifying/detecting one or more target nucleic acids in one or more biological samples.

95. [previously presented] The method according to claim 94 wherein said method produces an expression profile of one or more biological samples.

96. [previously presented] A method according to claim 57 for sequencing of one or more target nucleic acids of unknown sequence present in one or more biological samples, said method comprising the steps of:

(a) deriving from one or more biological samples one or more target nucleic acids in a single stranded form;

(b) subjecting the one or more target nucleic acids obtained from step (a) to a set of four separate base-specific complementary cleavage reactions, wherein each cleavage reaction generates a non-ordered set of fragments;

(c) analyzing the sets of non-ordered fragments obtained from step (b) by mass spectrometry;

(d) performing a systematic computational analysis on the mass spectra obtained from step (c) to assemble the sequence of said target nucleic acid; and,

(e) optionally, if the sequence is not uniquely defined after step (d), repeating steps (a) through (d), thereby generating modified forms of said target nucleic acid and/or different portions of said target nucleic acid, and performing supplementary mono- and/or di-nucleotide specific cleavage reactions rendering supplementary sets of non-ordered fragments until the combined data converge into a unique sequence solution,

wherein said complementary cleavage reactions refer to target nucleic acid digestions characterized by varying specificity and/or to digestion of alternative forms of the target sequence.

97. [canceled]

98. [canceled]

99. [previously presented] The method according to claim 92 wherein said method provides genome wide genotyping of one or more biological samples.

100. [previously presented] A kit for sequence analysis mass spectrometry re-sequencing according to a method of claim 57 89 of one or more target nucleic acids for which a reference nucleic acid sequence is known in one or more biological samples using mass spectrometry, the kit comprising:

(a) one or more nucleotide triphosphates;

(b) one or more polymerases;

(c) one or more nucleic acid cleaving agents; and;

(d) one or more sets of reference nucleic acids for which the nucleic acid sequence is known;

(e) reagents to purify the target nucleic acid;

(f) ion exchange beads in order to purify the non ordered set of fragments;

(g) a solid support suitable for use in mass spectrometry analysis whereon the non ordered set of fragments may be spotted; and,

(h) a computer software for comparing the mass spectra of the one or more target nucleic acid with the mass spectra of the reference nucleic acid and deducing therefrom the nucleic acid sequence of the target nucleic acid.

101. [canceled]

102. [previously presented] A kit for sequence analysis mass spectrometry sequencing according to a method of claim 57 of one or more unknown target nucleic acids in one or more biological sample using mass spectroscopy, the kit comprising:

(a) one or more nucleotide triphosphates;

(b) one or more polymerases; and,

(c) one or more nucleic acid cleaving agents;

(d) optionally, reagents to purify the target nucleic acid;

(e) ion exchange beads in order to purify the non ordered set of fragments;

(f) a solid support suitable for use in mass spectrometry analysis whereon the non ordered set of fragments may be spotted; and,

(g) computer software for analysing the mass spectra of the sequence of said target nucleic acid resulting in one or more unique sequences.

103. [canceled]

104. [previously presented] The method of claim 84, wherein the pyrimidine-specific RNase is RNase A.

105. [previously presented] The method of claim 57 or 89, wherein said method comprises four RNase-specific cleavage reactions.

106. [previously presented] The method of claim 105, wherein said four RNase-specific cleavage reactions comprise RNase T1 and RNase U2 cleavage of the + and – strands of said target nucleic acid.

107. [previously presented] The method of claim 105, wherein said four RNase-specific cleavage reactions comprise RNase A or RNase A and RNase T1 cleavage of the + and – strands of said target nucleic acid.

108. [previously presented] The kit of claim 100 wherein said cleaving agent is an endonuclease selected from the group consisting of U/C specific RNase A, G-specific T1 ribonuclease, A-specific U2 ribonuclease, A/U specific phyM ribonuclease, C-specific chicken liver ribonuclease (RNaseCL3) and cusativin.

109. [new] The kit of claim 102 wherein said cleaving agent is an endonuclease selected from the group consisting of U/C specific RNase A, G-specific T1 ribonuclease, A-specific U2 ribonuclease, A/U specific phyM ribonuclease, C-specific chicken liver ribonuclease (RNaseCL3) and cusativin.

110. [new] The kit of claim 100 or 102, wherein said one or more polymerases are SP6 and T7 RNA polymerase and said cleaving agent is an endonuclease selected from the group consisting of U/C specific RNase A, G-specific T1 ribonuclease, A-specific U2 ribonuclease, A/U specific phyM ribonuclease, C-specific chicken liver ribonuclease (RNaseCL3) and cusativin.

111. [new] The kit of claim 100, wherein said kit comprises:

(a) four nucleotide triphosphates;

(b) a T7 or SP6 polymerase;

(c) a RNase T1 and RNase U2;

(d) one or more sets of reference nucleic acids for which the nucleic acid sequence is known;

(e) reagents to purify the target nucleic acid;

(f) ion exchange beads in order to purify the non-ordered set of fragments;

(g) a solid support suitable for use in mass spectrometry analysis whereon the non-ordered set of fragments may be spotted; and

(h) a computer software for comparing the mass spectra of the one or more target nucleic acid with the mass spectra of the reference nucleic acid and deducing therefrom the nucleic acid sequence of the target nucleic acid.

112. [new] A kit of claim 102, wherein said kit comprises:

(a) four nucleotide triphosphates;

(b) a T7 or SP6 polymerase;

(c) a RNase T1 and RNase U2;

(d) reagents to purify the target nucleic acid;

(e) ion exchange beads in order to purify the non-ordered set of fragments;

(f) a solid support suitable for use in mass spectrometry analysis whereon the non-ordered set of fragments may be spotted; and,

(g) a computer software for analysing the mass spectra of the sequence of said target nucleic acid resulting in one or more unique sequences.

113. [new] The kit of claim 111 or 112, wherein said T7 or SP6 polymerase is a mutant polymerase that incorporates non-canonical substrates with a 2'-deoxy, 2'-O-methyl, 2'-fluoro or 2'-amino substituent into the transcript.

114. [new] A kit for mass spectrometry sequencing according to a method of claim 57 or claim 89 of one or more target nucleic acids in one or more biological samples using mass spectrometry, the kit comprising:

(a) one or more ribonucleotide triphosphates and one or more deoxyribonucleotide triphosphates;

(b) one or more polymerases;

(c) one or more RNAses; and

(d) a solid support suitable for use in mass spectrometry analysis whereon the non-ordered set of fragments may be spotted.